

## REMARKS

1. In reply to the objection raised in paragraphs 6 and 8 of the Office Action under U.S.C. §112, first paragraph, against claims 15-23, Applicants herewith submit a further Declaration concerning the deposit of the claimed yeast strain n° I-1032, executed by Mr Rossi, who is an authorized representative of the Assignee, LESAFFRE ET CIE, as well as the receipt of the original deposits and the corresponding translation into English (Attachment E).

Furthermore, the date of deposit for each deposit has been introduced into the specification.

2. From paragraph 9 of the Action, it appears that claims 7, 9, 10, 12, 14, 36, 38, 40, 41 and 51-59 are rejected under U.S.C. §112, first paragraph, the Examiner considering that the written description requirement is not satisfied.

Applicants respectfully disagree with the Examiner's position.

Amended claim 7 and new claims 61 and 62 were drafted to overcome the rejection.

In amended claim 7, the yeast strain of the fil phenotype has been identified by its survival rate, feature which can be evaluated without the necessity of the comparison with a reference strain.

Support for amended claim 7 is to be found from page 11 line 24 to page 12 line 2.

In new claims 60 and 61, the yeast strain of the fil phenotype has been identified by its stability against freezing in pieces of dough, feature which again can be evaluated without comparison with a reference strain.

Support for new claim 60 is to be found page 12, lines 3-11.

Support for new claim 61 is to be found page 12, lines 12 to 18.

Furthermore, it is to be emphasized that the specification discloses:

- several mutation methods resulting into the fil strains and fil strains thus obtained; in that respect please see in particular from page 17 line 25 to page 18 line 11 (screening tests), example 1, i.e. "Use of heat stress for the isolation of fil mutants" and example 2, i.e. "Use of the cycles of freezing / thawing for isolation of fil mutant from an industrial strain";

- in detail two specific mutations, one of which concerns the CYR1 gene, the other one concerning the GPR1 gene (see page 14, lines 1 to 6, and page 51, lines 22-30), as well as the use of these two mutations enabling in the construction of the new fil strains;
- in example 7, a means for the "Construction of new fil strains", whereby the mutation concerns several genes, and new fil strains thus obtained.

3. According to paragraph 10 of the Action, claims 2, 14, 57 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Applicants respectfully disagree with that rejection which does not take into consideration the general common knowledge of the skilled artisan. More particularly, it is well known by those skilled in the art that particular some yeast strains form, in an uncontrolled manner, secondary metabolites, the nature of which is often unknown, and which result in off-tastes and/or off-smells in breads.

When evaluating different yeast strain for their suitability for a given breadmaking process, it is conventional to prepare breads with said yeasts including and, after evaluation of said breads by a panel, to eliminate those yeasts which have produced metabolites which result in breads presenting an off-taste and/or an off-smell.

No strain is retained unless it successfully passes such an evaluation by a taste panel.

This evaluation is of particular importance when the construction of the yeasts is based on one or more mutations, which may involve genes other than the gene of interest.

This evaluation is of particular importance when the construction of the strains yeasts is based on a one or more mutations, which can may involve several genes in addition to the other than the geneone of interest. Some of these mutations are silent, i.e. without any apparent effect, while other mutations can may result into the formation of said undesired metabolites during breadmaking.

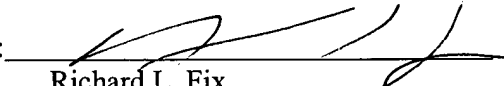
Consequently, the only solution, which is of a type conventionally used in the field of foodstuffs, is to prepare breads and to have their taste and smell evaluated by a panel.

Thus, for those skilled in the art, claims 2, 14, 57 and 58 are clear and definite.

In view of the newly presented claims, the attachments and the preceding arguments, it is believed that the application is now in condition for allowance and therefore reconsideration and allowance are respectfully requested.

Respectfully submitted,  
THEVELEIN et al

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Date

By:   
Richard L. Fix  
Reg. No. 28,297

STURM & FIX LLP  
206 Sixth Avenue, Suite 1213  
Des Moines, Iowa 50309-4076  
Phone: 515-288-9589  
Fax: 515-288-5311



**ATTACHMENT B**

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**Marked-up version of the claims**

1. A process for obtaining yeast strains conserving stress resistance in the presence of fermentable sugars, comprising the following steps:

a mutagenic treatment is carried out on the cells of a starting strain,

the cells having undergone the said mutagenic treatment are cultured so as to obtain a stationary phase,

the said cells in stationary phase are incubated in the presence of at least one fermentable sugar selected from the group comprising glucose, maltose, and sucrose, this sugar being present in a quantity such that the cells enter an active metabolic state (fermentation and/or growth),

said cells in active metabolic state are subjected to one or several stresses leading to a mortality rate of at least 99% with respect to the starting population,

the surviving cells are isolated and

those of the surviving cells which respond to the following criteria which characterize the fil phenotype are selected

- a growth, evaluated by production or production yield of biomass over sugar in a given time or by a growth rate, under identical culture conditions, at least equal to 80% of the starting strain,
- a CO<sub>2</sub> release, or a metabolite production, in identical conditions, at least equal to 80% of the starting strain,
- a stress resistance, corresponding to a survival rate at least 2 times higher than the survival rate of the starting strain, under identical phase conditions corresponding to a growth or active metabolism followed by a heat shock of at least 20 minutes at 52°C, or at least 1.5 times higher than the survival rate of the starting strain, under identical conditions of growth phase followed by freezing for a period of at least 24 hours at -20°C or at a lower temperature,
- maintenance of these properties after repeated cultures on non selective medium, so as to verify that the fil phenotype obtained by the mutation is perfectly stable and permanent.

2. A process according to claim 1, wherein it is checked that the selected yeast strains present an alcohol assimilation, under identical conditions, at least equal to 50% of that of the starting strain and that the selected yeast strains do not produce metabolites which give a bad smell or a bad or abnormal taste to breads.

3. A process according to claim 1, wherein the starting strain is an

industrial strain.

4. A process according to claim 3, wherein an industrial fil mutant carrying several mutations is obtained and wherein:

- the segregants issued from this industrial mutant are crossed with a laboratory haploid strain to select the segregant issued from this industrial mutant giving to the polyploids obtained with the laboratory strain an improvement in the required properties;
- the segregants thus selected are crossed one with the other;
- the polyploids obtained are selected according to the criteria of fil phenotypes defined in claim 1.

5. A process according to claim 1, wherein the selected fil strains have the property of conserving, in growth and/or fermentation phase on fermentable sugars, at least 50% of their survival rate with respect to the survival rate in stationary phase measured under the same conditions after a heat or freeze shock.

6. A process according to claim 1, wherein the cells obtained after mutagenic treatment are introduced into pieces of dough subjected to at least 100 cycles of freezing/thawing after a first fermentation of the dough of 30 minutes at 30°C.

7. (twice amended) An industrial yeast strain [having] of the fil phenotype having a survival rate, in growth phase on glucose, of at least 50% after heat treatment, the growth phase being defined as a cultivation of stationary cells on glucose for 10 minutes at 30°C after stationary phase.

9. A strain according to claim 7, belonging to *Saccharomyces cerevisiae* species.

10. A yeast strain according to claim 7 having a survival rate, in growth phase on fermentable sugars, of at least 50% after a heat treatment of 20 minutes at 52°C, the growth phase being defined as a reculturing on glucose of 10 minutes at 30°C after stationary phase.

12. An industrial yeast according to claim 7 whose stability to freezing in lumps of dough incubated 60 minutes at 30°C before freezing and containing 20 g of flour, 15 g of water, 1 g of sucrose, 0.405 g of NaCl, 0.06 g of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and 160mg of dry matter of the considered strain, defined by the ratio between the release of CO<sub>2</sub> at 30°C after 1 month or 30 days of conservation at -20°C and the release of CO<sub>2</sub> at 30°C after 1 day of conservation at -20°C, is at least equal to 80%.

14. A yeast strain according to claim 57, whose loss of released gas after drying of the biomass harvested in a phase close to exponential growth phase is at most equal to 67% of the

loss of released gas after drying of yeasts obtained using the corresponding starting strain.

15. (twice amended) Strain PVD1150 = M5 *fil* deposited at Collection Nationale de Cultures de Microorganismes (C.N.C.M.) under the n° I-2031 and the n° I-2203.
16. Strain KL1 = W303 *fil2* deposited at C.N.C.M. under the n° I-2032.
17. Strain FD51 = HL816 *fil300* deposited at C.N.C.M. under the n° I-2033.
18. Strain FDH16-22 = HL822 *fil300* deposited at C.N.C.M. under the n° I-2034.
19. Strain AT25 = S47 *fil400* deposited at C.N.C.M. under the n° I-2035.
20. Strain AT28 = S47 *fil500* deposited at C.N.C.M. under the n° I-2036.
21. Strain AT251 deposited at C.N.C.M. under the n° I-2222.
22. Strain AT252 deposited at C.N.C.M. under the n° I-2223.
23. Strain AT254 deposited at C.N.C.M. under the n° I-2224.
38. A dry baker's yeast obtained by culturing a strain according to claim 7.
40. A brewery yeast obtained by culturing a strain according to claim 7.
41. A yeast intended for the production of alcohol obtained by culturing a strain according to claim 7.
42. A process according to claim 1, wherein the yeast strains are of the *Saccharomyces cerevisiae* species.
43. ~~A process according to claim 1, wherein the selected yeast strains present a growth, evaluated by production or production yield of biomass over sugar in a given time or by a growth rate, under identical culture conditions, at least equal to 90% of the starting strain.~~
44. A process according to claim 1, wherein the selected yeast strains present a CO<sub>2</sub> release, or a metabolite production, in identical conditions, at least equal to 90% of the starting strain.
45. A process according to claim 1, wherein the selected yeast strains present a stress resistance, corresponding to a survival rate at least 3 times higher than the survival rate of the starting strain, under identical phase conditions corresponding to a growth or active metabolism followed by a heat shock of at least 20 minutes at 52°C, or at least 2 times higher than the survival rate of the starting strain, under identical conditions of growth phase followed by freezing for a period of at least 24 hours at -20°C or at a lower temperature.
46. A process according to claim 1, wherein the selected yeast strains present a stress resistance, corresponding to a survival rate at least 5 times higher than the survival rate of the starting strain, under identical phase conditions corresponding to a growth or active metabolism followed by a heat shock of at least 20 minutes at 52°C, or at least 3 times higher than the

survival rate of the starting strain, under identical conditions of growth phase followed by freezing for a period of at least 24 hours at -20°C or at a lower temperature.

47. A process according to claim 1, wherein the selected yeast strains present a stress resistance, corresponding to a survival rate at least 10 times higher than the survival rate of the starting strain, under identical phase conditions corresponding to a growth or active metabolism followed by a heat shock of at least 20 minutes at 52°C, or at least 5 times higher than the survival rate of the starting strain, under identical conditions of growth phase followed by freezing for a period of at least 24 hours at 20°C or at a lower temperature.

48. A process according to claim 1, wherein the selected fil strains have the property of conserving, in growth and/or fermentation phase on fermentable sugars, at least 60% of their survival rate with respect to the survival rate in stationary phase measured under the same conditions after a heat or freeze shock.

49. A process according to claim 1, wherein the selected fil strains have the property of conserving, in growth and/or fermentation phase on fermentable sugars, at least 70% of their survival rate with respect to the survival rate in stationary phase measured under the same conditions after a heat or freeze shock.

50. A process according to claim 1, wherein the selected fil strains have the property of conserving, in growth and/or fermentation phase on fermentable sugars, at least 80% of their survival rate with respect to the survival rate in stationary phase measured under the same conditions after a heat or freeze shock.

51. An industrial yeast strain according to claim 7 belonging to the *Saccharomyces* genus.

52. A yeast strain according to claim 7 having a survival rate, in growth phase on fermentable sugars, of at least 60% after a heat treatment of 20 minutes at 52°C, the growth phase being defined as a reculturing on fermentable sugar of 10 minutes at 30°C after stationary phase.

53. A yeast strain according to claim 7 having a survival rate, in growth phase on fermentable sugars, of at least 70% after a heat treatment of 20 minutes at 52°C, the growth phase being defined as a reculturing on fermentable sugar of 10 minutes at 30°C after stationary phase.

54. A yeast strain according to claim 7 having a survival rate, in growth phase on fermentable sugars, of at least 75% after a heat treatment of 20 minutes at 52°C, the growth phase being defined as a reculturing on fermentable sugar of 10 minutes at 30°C after stationary

phase.

55. An industrial yeast according to claim 7 whose stability to freezing in lumps of dough incubated 60 minutes at 30°C before freezing and containing 20 g of flour, 15 g of water, 1 g of sucrose, 0.405 g of NaCl, 0.06 g of  $(\text{NH}_4)_2\text{SO}_4$  and 160mg of dry matter of the considered strain, defined by the ratio between the release of  $\text{CO}_2$  at 30°C after 1 month or 30 days of conservation at -20°C and the release of  $\text{CO}_2$  at 30°C after 1 day of conservation at -20°C, is at least equal to 85%.

56. An industrial yeast according to claim 7 whose stability to freezing in lumps of dough incubated 60 minutes at 30°C before freezing and containing 20 g of flour, 15 g of water, 1 g of sucrose, 0.405 g of NaCl, 0.06 g of  $(\text{NH}_4)_2\text{SO}_4$  and 160mg of dry matter of the considered strain, defined by the ratio between the release of  $\text{CO}_2$  at 30°C after 1 month or 30 days of conservation at -20°C and the release of  $\text{CO}_2$  at 30°C after 1 day of conservation at -20°C, is at least equal to 90%.

57. An industrial yeast strain having the fil phenotype, obtainable by the process according to claim 1, presenting an alcohol assimilation, under identical conditions, at least equal to 50% of that of the starting strain and not producing metabolites which give a bad smell or a bad or abnormal taste to breads.

58. ~~A yeast strain according to claim 57, whose loss of released gas after drying of the biomass harvested in a phase close to exponential growth phase is at most equal to 50% of the loss of released gas after drying of yeasts obtained using the corresponding starting strain.~~

59. A baker's yeast obtained by culturing a yeast strain according to claim 7.

60. (new) An industrial yeast strain of the fil phenotype presenting a stability to freezing in pieces of dough containing 20g of flour, 15g of water, 1g of sucrose, 0.405g of NaCl, 0.06g of  $(\text{NH}_4)_2\text{SO}_4$  and an amount of the industrial yeast corresponding to 160mg of yeast dry matter, higher than 60%, said stability being defined as the ratio between the release of  $\text{CO}_2$  at 30°C after 30 days of conservation at -20°C and the release of  $\text{CO}_2$  at 30°C after 1 day of conservation at -20°C, whereby before freezing at -20°C, the pieces of dough are incubated at 30°C for 30 minutes.

61. (new) An industrial yeast strain of the fil phenotype presenting a stability to freezing in pieces of dough containing 20g of flour, 15g of water, 1g of sucrose, 0.405g of NaCl, 0.06g of  $(\text{NH}_4)_2\text{SO}_4$  and an amount of the industrial yeast corresponding to 160mg of yeast dry matter, higher than 80%, said stability being defined as the ratio between the release of  $\text{CO}_2$  at 30°C after 30 days of conservation at -20°C and the release of  $\text{CO}_2$  at 30°C after 1 day of conservation



at -20°C, whereby before freezing at -20°C, the pieces of dough are incubated at 30°C for 30 minutes.

Clean version of the replacement paragraphs**Specification, paragraph on page 13, lines 1-13**

- PVD1150 = M5 *fil* deposited at Collection Nationale de Cultures de Microorganismes (C.N.C.M.), 25 rue du Docteur Roux, F-75724 PARIS cedex, France, on May 27, 1998, under the n° I-2031 (contaminated strain) and on May 20, 1999 under the n° I-2203, in accordance with the criteria set forth in the Budapest Treaty.
- KL1 = W303 *fil*2 deposited at Collection Nationale de Cultures de Microorganismes (C.N.C.M.), 25 rue du Docteur Roux, F-75724 PARIS cedex, France, on May 27, 1998, under the n° I-2032, in accordance with the criteria set forth in the Budapest Treaty.
- FD51 = HL816 *fil*300 deposited at Collection Nationale de Cultures de Microorganismes (C.N.C.M.), 25 rue du Docteur Roux, F-75724 PARIS cedex, France, on May 27, 1998, under the n° I-2033, in accordance with the criteria set forth in the Budapest Treaty.
- FDH16-22 = HL822 *fil*300 deposited at Collection Nationale de Cultures de Microorganismes (C.N.C.M.), 25 rue du Docteur Roux, F-75724 PARIS cedex, France, on May 27, 1998, under the n° I-2034, in accordance with the criteria set forth in the Budapest Treaty.
- AT25 = S47 *fil*400 deposited at Collection Nationale de Cultures de Microorganismes (C.N.C.M.), 25 rue du Docteur Roux, F-75724 PARIS cedex, France, on May 27, 1998, under the n° I-2035, in accordance with the criteria set forth in the Budapest Treaty.
- AT28 = S47 *fil*500 deposited at Collection Nationale de Cultures de Microorganismes (C.N.C.M.), 25 rue du Docteur Roux, F-75724 PARIS cedex, France, on May 27, 1998, under the n° I-2036, in accordance with the criteria set forth in the Budapest Treaty.

**Specification, paragraph on page 29, lines 14-16**

Several mutants were obtained by the process according to the invention from the industrial polyploid strain S47 which is deposited at Collection Nationale de Cultures de Microorganismes (C.N.C.M.), 25 rue du Docteur Roux, F-75724 PARIS cedex, France,

on May 27, 1998, under the n° I 2037, in accordance with the criteria set forth in the Budapest Treaty.

**Specification, paragraph on page 58, lines 10-15**

AT251 strain was deposited at Collection Nationale de Cultures de Microorganismes (C.N.C.M.), 25 rue du Docteur Roux, F-75724 PARIS cedex, France, on June 10, 1999, under the n° I-2222, in accordance with the criteria set forth in the Budapest Treaty.

AT252 strain was deposited at Collection Nationale de Cultures de Microorganismes (C.N.C.M.), 25 rue du Docteur Roux, F-75724 PARIS cedex, France, on June 10, 1999, under the n° I-2223, in accordance with the criteria set forth in the Budapest Treaty.

AT254 strain was deposited at Collection Nationale de Cultures de Microorganismes (C.N.C.M.), 25 rue du Docteur Roux, F-75724 PARIS cedex, France, on June 10, 1999 under the n° I-2224, in accordance with the criteria set forth in the Budapest Treaty

Marked-up version of the replacement paragraphs

Underlined = inserted elements

[between square brackets and in bold print] = deleted elements

**Specification, paragraph on page 13, lines 1-13**

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  - KL1 = W303 *fil*2 deposited at Collection Nationale de Cultures de Microorganismes (C.N.C.M.), 25 rue du Docteur Roux, F-75724 PARIS cedex, France, on May 27, 1998, under the n° I-2032, in accordance with the criteria set forth in the Budapest Treaty.
  - FD51 = HL816 *fil*300 deposited at Collection Nationale de Cultures de Microorganismes (C.N.C.M.), 25 rue du Docteur Roux, F-75724 PARIS cedex, France, on May 27, 1998, under the n° I-2033, in accordance with the criteria set forth in the Budapest Treaty.
  - FDH16-22 = HL822 *fil*300 deposited at Collection Nationale de Cultures de Microorganismes (C.N.C.M.), 25 rue du Docteur Roux, F-75724 PARIS cedex, France, on May 27, 1998, under the n° I-2034, in accordance with the criteria set forth in the Budapest Treaty.
  - AT25 = S47 *fil*400 deposited at Collection Nationale de Cultures de Microorganismes (C.N.C.M.), 25 rue du Docteur Roux, F-75724 PARIS cedex, France, on May 27, 1998, under the n° I-2035, in accordance with the criteria set forth in the Budapest Treaty.
- AT28 = S47 *fil*500 deposited at Collection Nationale de Cultures de Microorganismes (C.N.C.M.), 25 rue du Docteur Roux, F-75724 PARIS cedex, France, on May 27, 1998, under the n° I-2036, in accordance with the criteria set forth in the Budapest Treaty.

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AT254 strain was deposited at Collection Nationale de Cultures de Microorganismes (C.N.C.M.), 25 rue du Docteur Roux, F-75724 PARIS cedex, France, on June 10, 1999, under the n° I-2224, in accordance with the criteria set forth in the Budapest Treaty.